



# MESOSCALE BIOTRANSFORMATIONS OF URANIUM IN SEDIMENTS AND SOILS (Program Element: Biogeochemistry)

Tetsu Tokunaga,<sup>1</sup> Jiamin Wan,<sup>1</sup> Eoin Brodie<sup>1</sup>, Yongman Kim<sup>1</sup>, Terry Hazen,<sup>1</sup>, Mary Firestone,<sup>2</sup>  
Don Herman,<sup>2</sup> Steve Sutton<sup>3</sup>, Matt Newville<sup>3</sup>, Tony Lanzirotti<sup>3</sup>, and Bill Rao<sup>4</sup>

<sup>1</sup>Lawrence Berkeley National Laboratory, <sup>2</sup>University of California, Berkeley, <sup>3</sup>University of Chicago, <sup>4</sup>University of Georgia

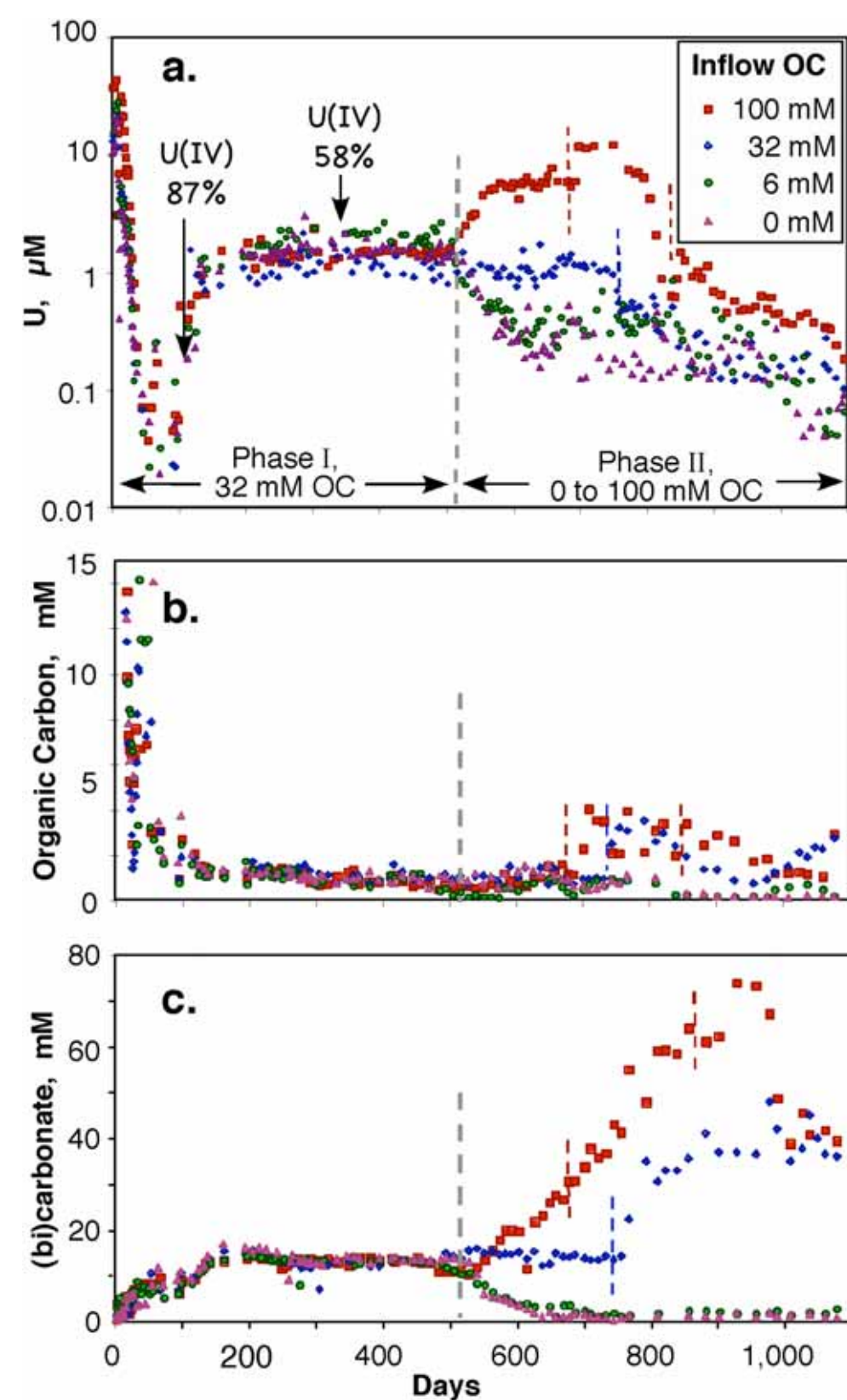


## Introduction

In-situ bioreduction is being considered as a remediation strategy for uranium (U) contaminated sediments because of its potentially low cost, and because short-term studies support its feasibility. However, any in-situ approach for immobilizing U will require assurance of either permanent fixation, or of very low release rates into the biosphere. Our long-term laboratory studies have shown that reoxidation of bioreduced  $\text{UO}_2$  can occur even under reducing (methanogenic) conditions sustained by continuous infusion of lactate. The biogeochemical processes underlying this finding need to be understood. Our current research is designed to identify mechanisms responsible for anaerobic U oxidation, and identify effects of key factors controlling long-term stability of bioreduced U. These include: (1) effects of organic carbon (OC) concentrations and supply rates on stability of bioreduced U, (2) influences of pH on U(IV)/U(VI) redox equilibrium, (3) the roles of Fe- and Mn-oxides as potential U oxidants in sediments, and (4) the role of microorganisms in U reoxidation. Findings from some of these studies are summarized here.

## I. Oxidation and Solubilization of Bioreduced $\text{UO}_2$ under Reducing Conditions

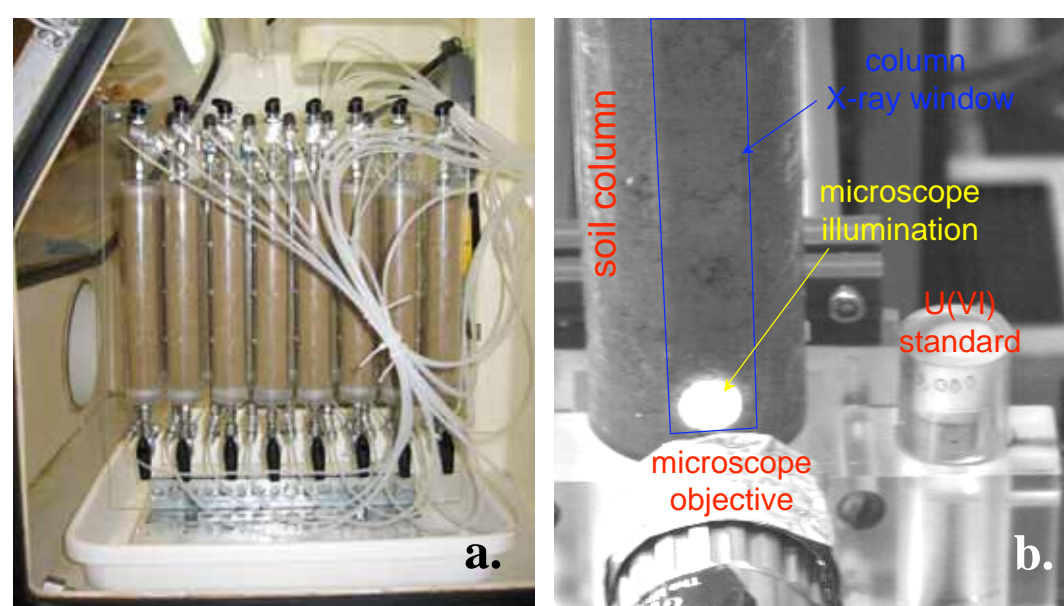
Part of our current work examines effects of varying influent OC concentrations on U mobility under reducing conditions. Through a long-term laboratory column experiment using FRC Area 2 soils, under continuous infusion of OC (lactate, at an OC concentration of 32 mM), our earlier study showed that U was reduced during the first 100 days, then reoxidized. These soil columns were subsequently infused with solutions having different OC concentrations (0 to 100 mM), beginning on Day 510. Rapid changes in effluent U concentrations occurred in response to these changes in OC supply. Both the 0 and 6 mM OC treatments yielded decreased U concentrations (contrary to conventional expectation), and the 100 mM OC treatment caused even higher levels of U in effluents (also contrary to conventional expectation). The system continuously supplied with 32 mM OC sustained a nearly steady outflow U concentration of about 1  $\mu\text{M}$ . These new results support our hypothesis that carbonate enrichment (from microbial oxidation of OC) promotes U(IV) oxidation because of the stability of U(VI) carbonate complexes, and also show that U-soil systems can be highly sensitive to OC supply. The terminal electron acceptor (TEA) driving U reoxidation is hypothesized to be a residual reactive Fe(III) fraction which becomes progressively depleted under sustained reduction. The steady decline in effluent U(VI) concentrations after about 700 days is indicative of TEA depletion.



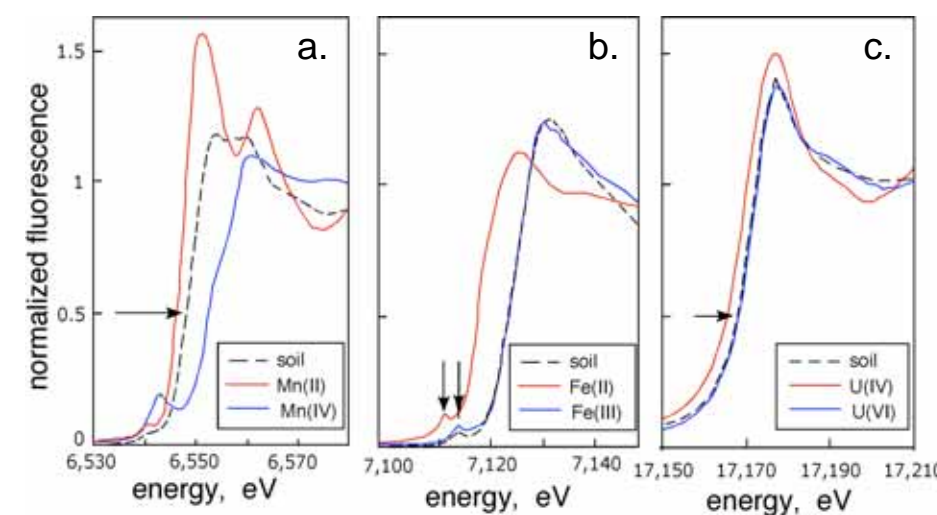
**Fig. 1.** Long term trends for (a.) U, (b.) OC, and (c.) (bi)carbonate in effluents from columns packed with historically U-contaminated sediment that were supplied with 32 mM OC (10 mM lactate) during Phase I, then switched to influents ranging from 0 to 100 mM OC (lactate) for Phase II. Major findings from Phase I were that effluent U concentrations increased back to high levels and U reoxidation occurred, even while reducing conditions were maintained. Our hypothesis that increased concentrations of U(VI) in effluents resulted from the stability of U(VI)-carbonate complexes and high levels of (bi)carbonate from OC mineralization was supported from measured high inorganic C concentrations, thermodynamic calculations, and direct measurements using laser fluorescence spectroscopy. This hypothesis was further supported by Phase II tests, where concentrations of influent OC were varied. Higher bicarbonate production from mineralization of higher OC influents released higher concentrations of U in effluents. At very long times (> 700 days), TEAs for U reoxidation are depleted, effluent U concentrations decline, and a net U reduction occurs. More recent experiments are testing the hypothesis that residual reactive Fe(III) is the TEA for U reoxidation, and that its in-situ depletion is very slow.

## II. Available terminal electron acceptors for U reduction under sustained reducing conditions

Several factors point to a residual reactive Fe(III) fraction in these sediments as the likely TEA for U reoxidation, and we are currently conducting experiments to further test this hypothesis. These include even longer-term column incubations targeted at completely reducing the reactive Fe(III) fraction in sediments, micro- X-ray absorption near-edge structure ( $\mu$ -XANES) spectroscopy for determining distributions of Mn, Fe, and U oxidation states in sediments at various stages of OC-stimulated bioreduction, and chemical methods for determining concentrations of Fe(II) and Fe(III). Results to date (110 days) continue to support the presence of a residual reactive Fe(III) fraction that can require years of sustained reduction to deplete.



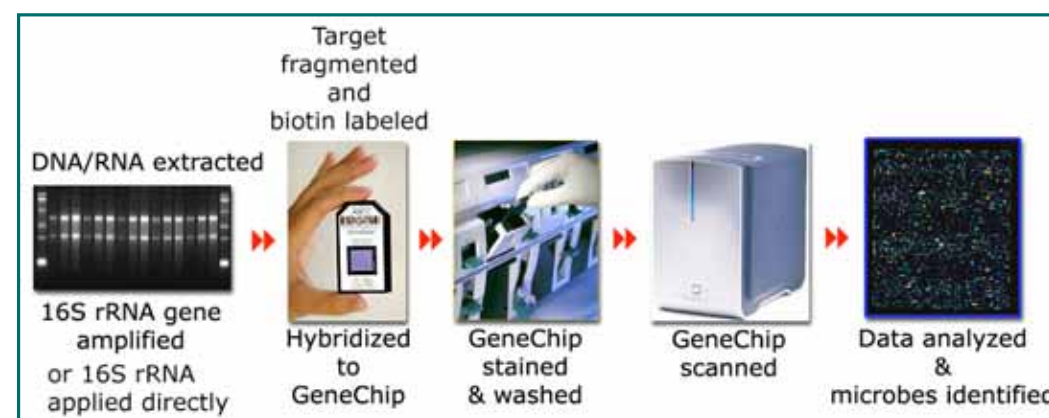
**Fig. 2.** Columns packed with uranium-contaminated sediment from FRC Area 2, Oak Ridge National Laboratory, (a.) in laboratory glovebox, being supplied with different OC solutions, (b.) on microprobe sample stage, in preparation for  $\mu$ -XANES spectroscopy measurements. X-ray microprobe beamlines at both APS (GSECARS 13ID-C) and NSLS (X26A) are being used to obtain direct, nondestructive, spatially- and temporally-resolved measurements of Mn, Fe, and U oxidation states in sediments.



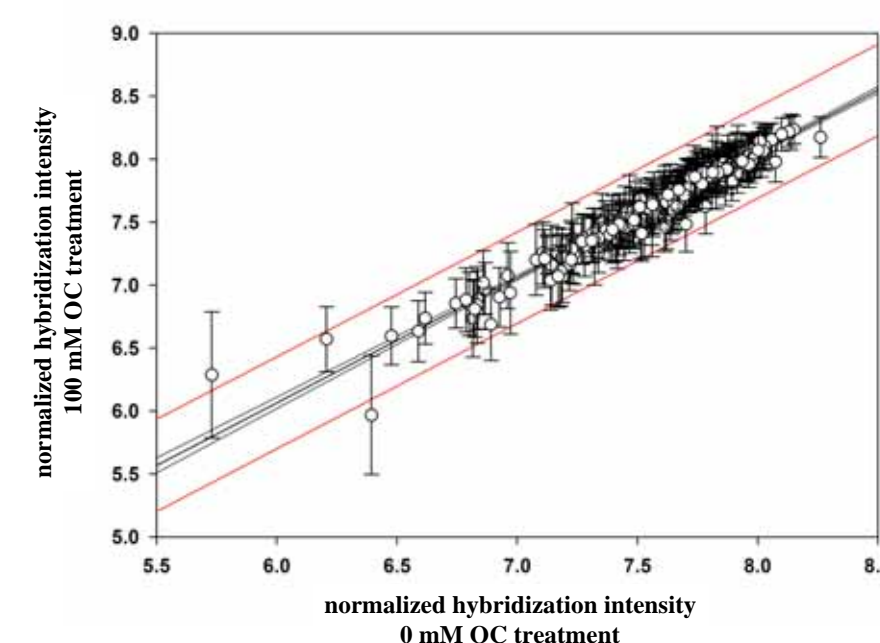
**Fig. 3.** Example  $\mu$ -XANES spectra obtained from one of many locations within a soil column. Reference marks denote diagnostic regions used for determining oxidation state. At this fairly early (38 days) stage of OC infusion, (a.) Mn is 65% reduced, (b.) Fe remains primarily (>95%) in Fe(III) forms, and (c.) U is still 95% ( $\pm 15\%$ ) U(VI).

## III. 16S PhyloChip analysis

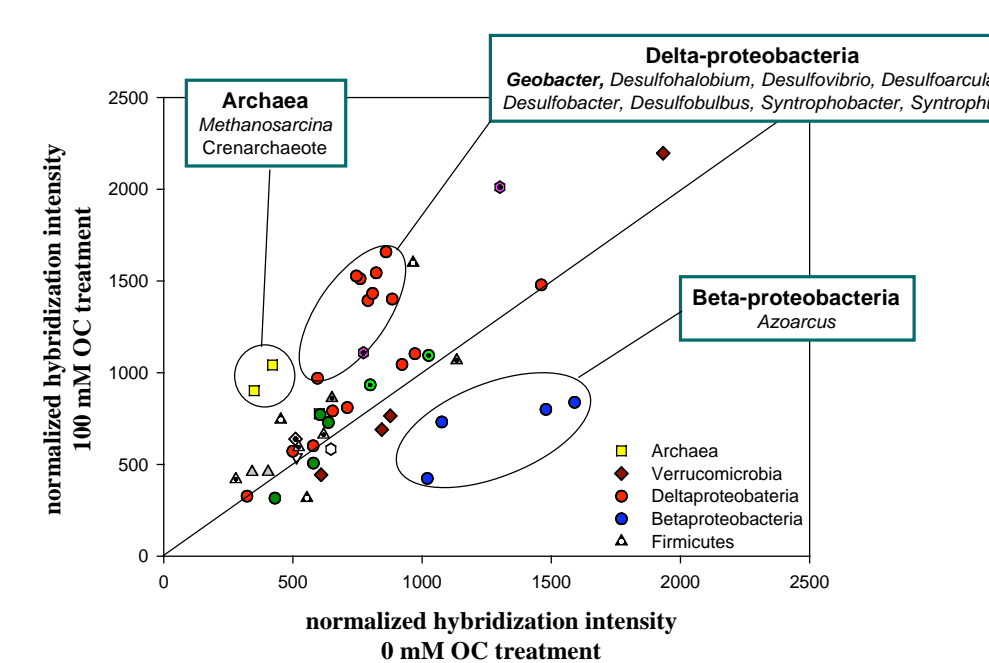
High-density 16S rRNA microarrays (PhyloChips) were used to monitor changes in bacterial community composition (16S PCR amplicons) and prokaryotic community activity (direct 16S rRNA hybridization).



**Fig. 4.** Schematic showing high-density DNA microarray (PhyloChip) approach. Either 16S rRNA gene amplicons (to follow changes in biomass) or 16S rRNA molecules (to follow changes in activity) can be labeled and hybridized to the array.



**Fig. 5.** Analysis of 16S PCR amplicons by PhyloChip. Columns receiving 100 mM OC show no significant differences in bacterial taxa abundance compared with columns receiving 0 mM OC after almost 200 days differential OC supply. In total ~8,200 bacterial taxa were assayed.



**Fig. 6.** Direct analysis of 16S rRNA molecules (active prokaryotes) by PhyloChip. Despite observed U remobilization, many delta-proteobacteria including *Geobacter* were more active in columns receiving 100 mM OC. An active population containing acetoclastic methanogens, sulfate reducers, iron reducers and the genera *Syntrophobacter* and *Syntrophus* strongly suggests syntrophy is occurring.

## Overall Summary

- Influent OC is largely mineralized to dissolved (bi)carbonate, including U(VI)-carbonate complexes.
- Thus,  $\text{U(VI)}_{\text{aq}}$  concentrations become proportional to influent OC concentrations while TEAs for U reoxidation remain available, even under sustained reducing conditions.
- Depletion of TEAs for U reoxidation can require years of sustained reduction, after which  $\text{U(VI)}_{\text{aq}}$  concentrations decline.
- $\mu$ -XANES spectra show rapid reduction of Mn(IV), and very slow reduction of Fe(III), indicating that reactive Fe(III) can persist over many hundreds of days under reducing conditions.
- PCR-independent direct rRNA analysis demonstrated the activity of U reducing bacteria despite observed remobilization and uncovered potential syntrophic interactions.
- Long-term experiments are needed to evaluate remediation strategies that rely on precipitating actinides and metals.

## Recent NABIR/EMSP Publications

- Zheng, Z., T. K. Tokunaga, and J. Wan. 2003. Influence of calcium carbonate on U(VI) sorption to soils. *Environ. Sci. Technol.* 37, 5603-5608.
- Wan, J., T. K. Tokunaga, J.T. Larsen, and R. J. Serne, 2004. Geochemical evolution of highly alkaline and saline tank waste plumes during seepage through vadose zone sediments. *Geochim. Cosmochim. Acta.* 68, 491-502.
- Wan, J., J.T. Larsen, T.K. Tokunaga, Z. Zheng, 2004. pH neutralization and zonation in alkaline-saline tank waste plumes. *Envir. Sci. Technol.* 38, 1321-1329.
- Tokunaga, T.K., J. Wan, J. Pena, S.R. Sutton, and M. Newville. 2004. Hexavalent uranium diffusion in soils from concentrated acidic and alkaline solutions. *Environ. Sci. Technol.* 38, 3056-3062.
- Wan, J., T.K. Tokunaga, E. Brodie, Z. Wang, Z. Zheng, D. Herman, T.C. Hazen, M.K. Firestone, and S.R. Sutton. 2005. Reoxidation of bioreduced uranium under reducing conditions. *Environ. Sci. Technol.* 39, 6162-6169.
- Tokunaga, T.K., J. Wan, J. Pena, E. Brodie, M. Firestone, T. Hazen, S. Sutton, A. Lanzirotti, and M. Newville. 2005. Uranium reduction in sediments under diffusion-limited transport of organic carbon. *Environ. Sci. Technol.* 39, 7077-7083.

## Acknowledgments

We thank J. Larsen, T. DeSantis, G. Andersen, D. Joyner, and S. Baek for experimental support, and the U.S. Department of Energy, Environmental Remediation Sciences Program for support of this research. Dr. David Watson and the FRC staff generously provided information and sediment samples. Some of this work was conducted at the Advanced Photon Source through support from Basic Energy Sciences, Office of Science, and at the National Synchrotron Light Source with support from the Division of Materials Science and Division of Chemical Sciences.